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ABSTRACT (Maximum 200 words)

These studies examined chiral reactivity, the magnitude and importance of steric interactions, and the importance of the electrostatic charge distribution in governing reactivity of acetylcholinesterase (AchE). To do this, we employed a comprehensive series of resolved enantiomeric methylphosphonates, a comprehensive series of alkylphosphonates, and fluorescent methylphosphonates. As a complementary index to detailed stopped-flow analysis of inhibition kinetics, we monitored kinetics of aging and oxime reactivation. Together with equilibrium binding studies, employing the reversible fluorescent ligand decidium diiodide, these provided an independent index of ligand orientation achieved by the organophosphonates within the enzyme active center.

14. SUBJECT TERMS
Organophosphonates; Methylphosphonates; Enantiomers; Fluorescence spectroscopy; Acetylcholinesterase; Aging; Oxime reactivation; Bimolecular inhibition.

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Nowak, M. W. and Berman, H. A. (1995) Influence of electrolytes on conformation of acetylcholinesterase" Biochemistry (submitted, June, 1995).

Berman, H.A. (1995) "Covalent Reactions of Acetylcholinesterase: Molecular fate at the rim of a gorge" IN *Vth International Conference on the Cholinesterase: Structure, function, and regulation.* Eds. Quinn, D, M. and Doctor, B. P. American Chemical Society Press, Washington, D.C. (in press).

Hosea, N., Berman, H. A., and Taylor, P. (1995) Specificity and orientation of trigonal carboxyl esters and tetrahedral alkylphosphonyl esters in cholinesterases. Biocchemistry (in press).

8. SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT AND DEGREES AWARDED DURING THIS REPORTING PERIOD:

Shafqat Fazili, Research Technician
Kristie A. Harms, Research Technician
Mark W. Nowak, PhD student, degree awarded May, 1992
Zhigong Luo, PhD student, degree awared April, 1993
Mary Stock, M.D. student (Summer, 1992)
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9. REPORT OF INVENTIONS (BY TITLE ONLY): No inventions to report.

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BRIEF OUTLINE OF RESEARCH FINDINGS.

As indicated by our previous reports and the titles of the above submitted papers and that accepted for publication, research in our laboratory has pursued two distinct areas of cholinesterase structure and function. One area concerns catalysis by acetylcholinesterase (AchE), and mechanisms governing covalent reactivity of the enzyme. These endeavors complement our larger studies on reaction of AchE with structurally-related organophosphonates and subsequent reactivation of the corresponding conjugates. One paper, in collaboration with the P. Taylor lab, examined the role played in ligand specificity by two phenylalanine residues within the active center. Employing site-directed mutant strains of mouse AchE, in conjunction with purified and resolved enantiomeric methylphosphonates we have synthesized, it is found that F297 plays a major role in governing enzyme chiral specificity. Furthermore, in studies of purified *Torpedo* AchE, we find through examination of a comprehensive number of alkylphosphonates, that ligand orientation is governed by charge as well as interactions at the mouth of the active center gorge. That is to say, that at the mouth of the gorge, alkylphosphonates – dependent on whether they are charged or uncharged – undergo interactions the strength of which determines the eventual rate of reaction as well as the eventual chiral outcome. Finally, Mark Nowak, a recent graduate from my lab, has found that while ligand specificity is governed in part by ligand structure, the enzyme is highly labile and undergoes readily labile distortions in conformation with subtle changes in ionic composition of the medium. of particular curious interest is the finding that AchE displays a unique capacity to recognize not only changes in ionic strength, but also ionic composition, in that the operational conformation changes in one direction in the presence of monovalent alkali ions (Na^+, K^+) and in a unique direction in the presence of divalent alkaline earth ions (Mg^{++}, Ca^{++}).

These latter studies represent a logical connection with those we have previously reported to you, some of which remain in near-final states of preparation. These concerned the influence of THA, the anti-Alzheimer drug that is reported to augment central cholinergic function. As we mentioned in an earlier report, THA associates with AchE at the active center and with very high affinity (3-10 nM), behaviour is consonant with the intuitive mechanism that the therapeutic efficacy of THA reflects its capacity to inhibit AchE, thereby elevating synaptic concentrations of acetylcholine and, in turn, enhancing cholinergic activity. However, we have found upon *cellular* examination of the action of THA in neuron that such a mechanism was too simple to explain such therapeutic efficacy, in that *THA caused a marked increase, in the range 300-400 %, in cellular amounts of AchE*. The mechanism of these actions continues to be under study.